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SERIAL NO. 09/389,565

APPENDIX B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Neville *et al.*

Serial No. 09/389,565

Filed: September 3, 1999

For: "AN IMMUNOTOXIN WITH IN VIVO T CELL SUPPRESSANT ACTIVITY AND METHODS OF USE"

Examiner: G. R. Ewoldt, Ph.D.

Art Unit: 1644

DECLARATION OF DAVID M. NEVILLE, JR. UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Washington DC 20231

NEEDLE & ROSENBERG, P.C.
The Candler Building
127 Peachtree Street, N.E.
Atlanta, Georgia 30303-1811

Examiner Ewoldt:

I, David M. Neville, Jr., a citizen of the United States, residing at 9624 Parkwood Drive, Bethesda, Maryland 20814, declare that:

1. A single-chain recombinant antibody fusion protein derived from the monoclonal antibody UCHT1 and a truncated diphtheria toxin (DT) has surprising and unexpected properties; such as, a high relative potency when compared to other anti-CD3 monoclonal antibodies in

the context of truncated DT immunotoxins (ITs). These surprising and unexpected properties arise from characteristics of the sFv moiety of UCHT1 that are not present in most other sFv constructs of other anti-CD3 antibodies and also arise from the unique synergy between the truncated DT and the CD3ε epitope.

2. Potency assays were conducted in which a reference sample, either a chemical conjugate of intact UCHT1-CRM9 (CRM9 being a DT binding site mutant) or the single chain ITs DT390sFv(UCHT1) or DT389sFv(UCHT1) were compared to other single-chain ITs made with the sFvs of other anti-CD3 antibodies. The potency assays were either protein synthesis inhibition assays or assays of the inhibition of viable cell growth based on the vital dye MTS. $CD3^+$ Jurkat cells were used for all assays of the anti-human CD3 antibodies UCHT1, CRIS7 and SP34. Monkey primary cultured T cells were used for the anti-rhesus monoclonal antibody, FN18. All these assays are performed with 6 replicates of each data point.

3. Potency assays comparing the chemical conjugate of an intact antibody, FN18-CRM9, with the recombinant derivative, DT390sFv(FN18), showed that the recombinant IT was 72-fold less potent than the chemical conjugate (Exhibit A). The data of Exhibit A indicate that ITs had greatly reduced potency relative to that of chemical conjugates. By contrast, Exhibit B shows that there was no change in potency between a chemical conjugate of UCHT1-CRM9 and the single-chain recombinant IT, DT390sFv(UCHT1). This observation was remarkable, not only because the UCHT1-CRM9 has two variable regions (i.e., 2 VH and two VL regions) whereas DT390sFv(UCHT1) has only one (I.e., one VH and one VL region), but also because a similar result was not observed with FN18 (Exhibit A). These data suggested that the sFv of FN18 lacked an essential property that is present in the sFv of

UCHT1 which affected the potency of the IT. To see if this property was shared among other antibodies, the potency of the chemical conjugate UCHT1-CRM9 was compared to the recombinant IT DT390sFv(SP34) (Exhibit C). DT390sFv(Sp34) was decreased in potency by 70-fold compared the chemical conjugate. Similarly a comparison of DT389sFv(CRIS7) and DT390sFv(UCHT1) was conducted which showed that the DT389sFv(CRIS7) IT was down 42-fold in potency over the similar IT constructed with the sFv of UCHT1 (Exhibit D). These data suggested that the SP34 sFv and CRIS7 sFv lacked an important property necessary for high potency that was present in the sFv of UCHT1.

4. A potential explanation for a low potency of a non-UCHT1 recombinant IT compared to a UCHT1 recombinant IT was that the non-UCHT1 parental antibody was not as high in affinity as UCHT1. Exhibit E shows that the potency of a chemical conjugate made with the SP34 antibody and CRM9 was within 2.2-fold the potency of UCHT1-CRM9. Thus the marked difference in potency between the recombinant IT was not caused by a difference in affinity of parental antibodies. Note that the Exhibit E data were obtained in a 5-hour assay, which shifts the dose response curves to the right preventing direct comparisons of IC-50 potencies between 5 and 20 hour assays. Thus, in these experiments a standard IT was always provided, either UCHT1-CRM9 or DT390sFv(UCHT1) or the 389 analog), which all have equal potencies. In Exhibit F, FACS competition data showed that the affinity of the monoclonal antibody CRIS7 was 9.5-fold higher than the monoclonal antibody UCHT1. This result indicated that the low potency of DT389sFv(CRIS7) compared to DT389sFv(UCHT1) in Exhibit D was not explained by a lower affinity of the parental CRIS7

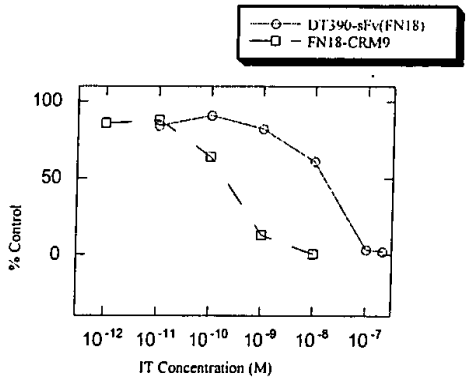
antibody compared to UCHT1. Rather, the explanation must be sought in the difference in the properties of the respective sFvs.

5. The unpredictable differences between various sFvs with respect to specific binding and specific T cell toxicity when present in fusion ITs may reflect the varying stability of the sFv to maintain its structure in the face of neighboring steric hindrance. Such instability may result in altered binding pockets and, therefore, altered ability to bind the target. However, the state of the art at the time of the invention, and even now, was such that one skilled in the art could not predict the relative success of a binding pocket of a given sFv moiety when placed carboxy-terminal to DT390 in a fusion protein.

6. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.

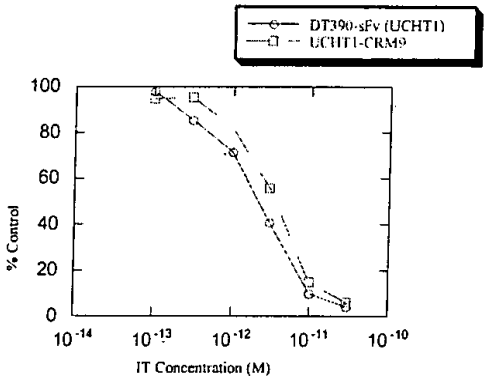
2-10-03
Date

David M. Neville Jr.
David M. Neville, Jr.



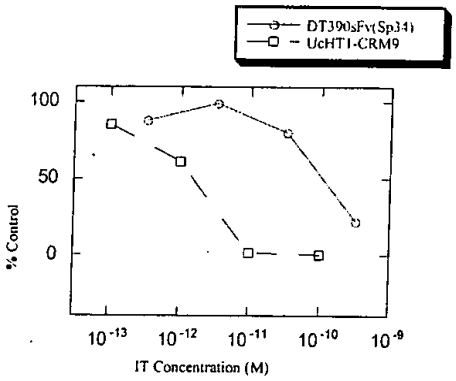
Cytotoxicity Assay (20 hrs.) of the single chain immunotoxin DT390-sFv(FN18) compared to chemicaly conjugated FN18-CRM9 on monkey primary T cells by protein synthesis inhibition.

Exhibit A



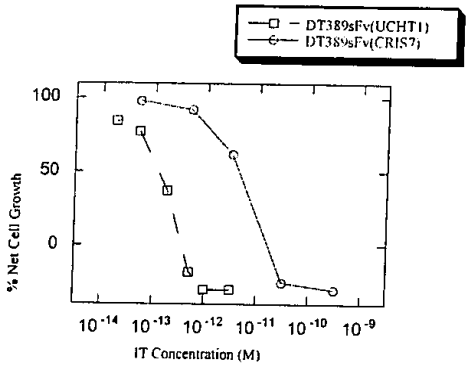
Cytotoxicity Assay (20 hrs.) of the single chain immunotoxin DT390sFv(UCHT1) compared to the chemical conjugate UCHT1-CRM9 on Jurkat cells by protein synthesis inhibition. Note that the potencies are essentially equal.

Exhibit B



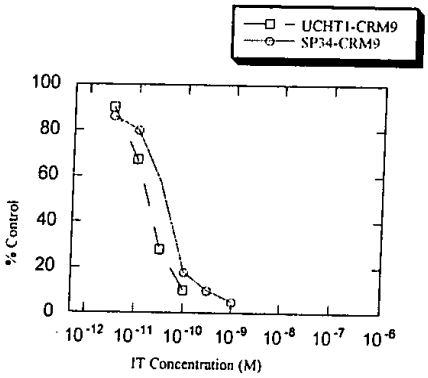
Cytotoxicity Assay (20 hrs.) of the single chain immunotoxin DT390-sFv(Sp34) compared to chemicaly conjugated UCHT1-CRM9 on Jurkat cells by protein synthesis inhibition.

Exhibit C



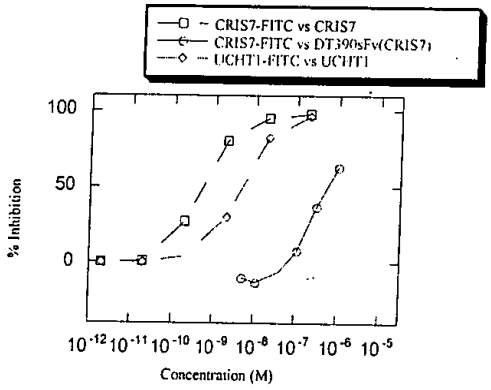
Cytotoxicity Assay of the single chain immunotoxins DT389-sFv(UCHT1) and DT389-CRIS7 by MTS dye incorporation into viable Jurkat cells

Exhibit D



Cytotoxicity Assay (5 hrs.) of the chemical conjugate SP34-CRM9 compared to the chemical conjugate UCHT1-CRM9 on Jurkat cells by protein synthesis inhibition.

Exhibit E



Competitive FACS analysis was used to determine the affinity of the mAbs CRIS7 and UCHT1 and the IT DT389sFv(CRIS7) yielding affinities of 1.8×10^{-9}, 1.9×10^{-8} and 1.6×10^{-6} respectively.

Exhibit F